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Strong association between glucocerebrosidase mutations and Parkinson's disease in Sweden

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ABSTRACT

Several genetic studies have demonstrated an association between mutations in glucocerebrosidase (*GBA*), originally implicated in Gaucher's disease, and an increased risk of Parkinson's disease (PD). We have investigated the possible involvement of genetic *GBA* variations in PD in the Swedish population. Three *GBA* variants, E326K, N370S, and L444P were screened in the largest Swedish Parkinson cohort reported to date; 1625 cases and 2025 control individuals. We found a significant association with high effect size of the rare variant L444P with PD (odds ratio 8.17; 95% confidence interval: 2.51–26.23; *p*-value = 0.0020) and a significant association of the common variant E326K (odds ratio 1.60; 95% confidence interval: 1.16–2.22; *p*-value = 0.026). The rare variant N370S showed a trend for association. Most L444P carriers (68%) were found to reside in northern Sweden, which is consistent with a higher prevalence of Gaucher's disease in this part of the country. Our findings support the role of *GBA* mutations as risk factors for PD and point to lysosomal dysfunction as a mechanism contributing to PD etiology.

1. Introduction

The human glucocerebrosidase gene (*GBA*) is located on chromosome 1q21 and encodes a lysosomal protein, which cleaves the beta-glycosidic bond of glucosylceramide, an intermediate formed during glycolipid metabolism (Brady et al., 1965). Mutations in *GBA*, originally implicated in Gaucher's disease (GD), have been identified both in familial and sporadic Parkinson's disease (PD; Balicki and Beutler, 1995; Lwin et al., 2004). These mutations also seem to influence the risk of developing Lewy body dementia and Lewy body dementia with Alzheimer-like neuropathologic changes, but do not associate with Alzheimer's disease (Clark et al., 2009; Tsuang et al., 2012). Genetic alterations of *GBA*, α-synuclein (*SNCA*), and leucine-rich repeat kinase 2 (*LRRK2*) together constitute the most common known genetic risk-factors for sporadic PD today (Ran and Belin, 2014).

Genetic variations in *GBA* are known to alter glucocerebrosidase (GCase) activity and decreased GCase activity, and protein levels have repeatedly been reported in brain tissue from PD patients with *GBA* mutations (Gegg et al., 2012; Lwin et al., 2004; Mazzulli et al., 2011). Interestingly, PD patients without *GBA* mutations were recently reported to have lower GCase activity as compared with healthy controls, suggesting *GBA* is involved in the pathologic mechanisms of PD even in the absence of known *GBA* gene

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mutations (Alcalay et al., 2015; Chiasserini et al., 2015; Murphy et al., 2014). The most frequent mutations in *GBA* reported to be associated with increased risk of PD are L444P and N370S (Sidransky et al., 2009). The associations of these 2 mutations with PD have been observed in populations with diverse genetic backgrounds and represent over 50% of the pathogenic *GBA* mutations found in patients globally (Sidransky et al., 2009). These findings were verified in a large European study comprising almost 1400 sporadic and familial PD patients, where L444P or N370S were found in 70% of the *GBA* mutation carriers (Lesage et al., 2011). Many other *GBA* mutations have been reported to influence the risk of developing PD, and the frequency of each variant differs globally according to genetic background (Gan-Or et al., 2015; Sidransky et al., 2009). In particular, *GBA* mutations are more common in subjects with Ashkenazi Jewish ancestry (Aharon-Peretz et al., 2004; Gan-Or et al., 2008; Sidransky et al., 2009).

Not only rare mutations but also single nucleotide polymorphisms (SNPs) such as E326K have been suggested to affect the risk of developing PD (Clark et al., 2007; Lwin et al., 2004). E326K has been reported to associate with PD in European populations, although there are conflicting results (Lesage et al., 2011; Sidransky et al., 2009). The association with E326K is particularly strong in PD patients with early disease onset and suggested to result in an aggravated phenotype with cognitive decline (Duran et al., 2013; Mata et al., 2015). GCase activity measured in post-mortem brains of PD patients heterozygous for E326K ranged between 90% and 100% of the activity in wild-type carriers (Lwin et al., 2004), but in combination with other *GBA* mutations, for example L444P, the E326K mutation leads to further deterioration of enzymatic activity (Chabas et al., 2005; Montfort et al., 2004).

GBA mutations have not frequently reached significance in genome-wide association studies (GWAS), probably because of the low minor allele frequencies of PD associated *GBA* mutations and the methodological difficulties in avoiding contaminating signals from the pseudogene *GBAP1* (glucosidase, beta, acid pseudogene 1). Other factors that might influence the outcome of a GWAS are which genetic markers are included and how these are linked to disease associated variants. Nevertheless, 2 GWAS studies published in 2011 and 2012 reported association between PD and N370S, and one of them further supports the E326K association (Do et al., 2011; Pankratz et al., 2012). These data have also been confirmed in large meta-analyses (Lill et al., 2012; Nalls et al., 2014).

Parkinson patients carrying heterozygous *GBA* mutations are characterized by relatively early disease onset, but are otherwise clinically similar to patients with sporadic PD and respond well to L-DOPA (Clark et al., 2007). However, patients with *GBA* mutations have been reported to be more likely to suffer from nonmotor symptoms, in particular cognitive impairment and dementia (Alcalay et al., 2012; Mata et al., 2015; Seto-Salvia et al., 2012). To gain insight into the role of *GBA* in the etiology of PD in the relatively homogenous Swedish population, we investigated the presence of 3 most reported nonsynonymous *GBA* variants in PD, N370S, L444P, and E326K, among Swedish Parkinson patients and controls. In the light of the particularly elevated prevalence of *GBA* mutations in individuals with Ashkenazi Jewish descent, the Swedish population, which has not previously been screened for GBA mutations, is especially interesting to study as there is a known occurrence of GD type III (Norrbottnian type) in northern Sweden (Dahl et al., 1990).

2. Materials and methods

2.1. Human DNA and tissue

A total of 1625 Swedish PD patients were recruited from the neurology clinics at Karolinska University Hospital, Stockholm, Sahlgrenska University Hospital, Gothenburg, Skåne University Hospital, Lund, Umeå University Hospital, Umeå and Linköping University Hospital, Linköping. Patient material was obtained after informed consent and approval of the local ethics committees in Stockholm, Gothenburg, Lund, Umeå, and Linköping, respectively (http://www.epn.se). Information on cognitive decline was only available for patients from Lund and was reported for 33 of the 122 patients (27.9%) which were also included in the genetic screening. The PD patients were diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank criteria for idiopathic PD, including patients who declared having one or more first, second, or third degree relatives with PD to get a large material (Gibb and Lees, 1988). The 2025 Swedish control subjects were recruited from the corresponding catchment areas as the patient material and consisted of spouses of PD patients, individuals visiting the neurology clinic, blood donors, and subjects recruited from an ongoing longitudinal study, SNAC-K (The Swedish National Study on Aging and Care in Kungsholmen, http://www.snac-k.se/), see Table 1 for further site specific demographic information. Screenings of known PD mutations in *LRRK2* and *SNCA* have been performed previously on a subset of the joint case-control material (Carmine Belin et al., 2006; Puschmann, 2011; Westerlund et al., 2008). All subjects were unrelated and a majority of Caucasian origin (>95%). DNA was extracted from whole blood according to standard protocols.

2.2. Genotyping

2.2.1. Pyrosequencing

The 3 genetic *GBA* variants were genotyped by pyrosequencing (Ronaghi et al., 1998), except for a fraction of samples which were genotyped for N370S with TaqMan (see paragraph 2.2.2 on TaqMan SNP genotyping for details). Primer sequences are available on request (Thermo Scientific, MA, USA). Primers were designed using free online software (Primer 3 v4.0.0 and mFold v3.6; Koressaar and

Table 1
Demographic data for the Parkinson patients and control populations

Variable	Gothenburg		Linköping		Lund		Stockholm		Umeå	
	PD	CTRL	PD	CTRL	PD	CTRL	PD	CTRL	PD	CTRL
Individuals, *n*	171	190	195	366	122	43	528	1271	609	155
Mean age at enrollment, y	68.2	69.1	71.4	70.1	69.9	67.9	67.4	71.6[b]	68.9	64.1
Mean age at diagnosis, y	59.0	NA	63.6	NA	61.9	NA	58.8[a]	NA	62.9	NA
Females, *n* (%)	74 (43.3)	120 (63.2)	74 (38.0)	185 (50.5)	48 (37.7)	27 (62.8)	193 (36.55)	631 (49.9)	225 (36.9)	77 (49.6)
Heredity, *n* (%)	41 (24.0)	NA	42 (21.5)	NA	68 (54.8)	NA	125 (36.8)[a]	NA	121 (19.9)	NA

Heredity was defined as having one or more first, second, or third degree relatives with PD.
Key: CTRL, control subjects; NA, data not applicable; PD, Parkinson's disease.
[a] Calculation based on 355 individuals for whom this information was available.
[b] Calculation based on the 416 individuals for whom this information was available, the remaining 855 controls were blood donors.

Remm, 2007; Untergasser et al., 2012; Zuker, 2003). One of the primers in each primer-pair was biotinylated at the 5'-end to allow subsequent immobilization to streptavidin-coated beads. All primers were designed to bind specifically to *GBA* by targeting parts of the sequence that varied between *GBA* and the pseudogene *GBAP1*. A polymerase chain reaction (PCR) reaction was run for 45 cycles at 95 °C for 20 seconds, annealing for 20 seconds at different temperatures: 57 °C for N370S (rs76763715), and 60 °C for L444P (rs421016) and E326K (rs2230288). After PCR, the biotinylated PCR product was immobilized to streptavidin-coated beads (GE Healthcare, Buckinghamshire, United Kingdom), mixed for 10 minutes at room temperature and captured onto filter probes (PyroMark Vacuum Prep Tool, Qiagen, Venlo, the Netherlands). The filter probes were flushed with 70% ethanol, 1M NaOH denaturation solution, and washing buffer and the single-stranded template was annealed to the sequencing primer at 80 °C for N370S, L444P, and 90 °C for E326 K for 2–3 minutes. All solutions used in sample preparation were made according to manufacturer's instructions (QIAGEN). Samples were analyzed on an automated pyrosequencer (PSQ 96 System with SNP Software and PyroMark GOLD Reagent Kits; QIAGEN).

2.2.2. TaqMan SNP genotyping

Two hundred forty-nine samples from Karolinska University Hospital, 165 samples from Skåne University Hospital, Lund, and 854 samples from Umeå University were genotyped for N370S using TaqMan SNP genotyping (ABI 3730 DNA Analyzer instrument; Applied Biosystems, Carlsbad, CA, USA). We used a custom-designed assay (forward primer: 5′ GTG ACC CTT ACC TAC ACT CTC T, reverse primer: 5′ GGT TCA GGG CAA GGT TCC), the reverse primer being specific for the *GBA* gene, thus excluding the pseudogene *GBAP1*. Allelic discrimination was run with 2 5′ fluorescence-labeled allele-specific probes: C_2286559_30 and C_2268560_20, genotyping master mix (TaqMan, Applied Biosystems), and 20 ng of genomic DNA. We used the default PCR fast cycling conditions, 10 minutes at 95 °C, 15 seconds at 92 °C, and 1 minute at 60 °C, for 40 cycles. Analysis was performed with software supplied with the instrument (QuantStudio 6 and 7, Flex Real-Time PCR System Software v1.0). Mutation carriers detected with TaqMan were resequenced by pyrosequencing to confirm results.

2.3. Statistical analysis

We achieved a call rate of 95.3% for E326K, 95.8% for N370S, and 97.4% for L444P. Genotypic and allelic associations were evaluated separately for southern and northern Sweden because of uneven geographical distribution of mutation carriers. Analysis was performed under a dominant model, using appropriate software (Prism 5.03; GraphPad Softwares Inc, La Jolla, CA, USA) with chi-square (χ^2) and Fisher's exact test, significance level 5%, 2-sided *p*-values. Results were compared between southern and northern Sweden by means of a meta-analysis based on the odds ratio (ORs) obtained from the dominant genotype analysis. Meta-analysis included Cochran's Q statistics and calculation of the heterogeneity index I^2 as a measure of consistency between studies and was performed in PLINK v1.07 (Purcell et al., 2007). The meta-analysis was run under a random-effects model. Bonferroni correction for multiple testing was used in both the association analysis and the meta-analysis. We used a χ^2 Hardy-Weinberg equilibrium (HWE) test to calculate HWE for each variation in the control population (Rodriguez et al., 2009). To compare demographic characteristics between groups, unpaired Student's *t* test was used; 2-tailed *p*-values (Prism 5.03).

3. Results

3.1. Association analysis

Our study included 3 genetic variations in *GBA*, 2 rare mutations: N370S and L444P, and the more common SNP, E326K. Parkinson patients and healthy control individuals from 5 different geographic locations in Sweden were analyzed. We found that the 2 rare variants occurred more often in PD patients than in healthy individuals (Table 2). Furthermore, E326K was overrepresented in PD patients, with a minor allele frequency of 3.05%, versus 1.75% in controls. None of the identified *GBA* mutation carriers have been reported to carry *SNCA* and/or *LRRK2* mutations to date (Carmine Belin et al., 2006; Puschmann, 2011; Westerlund et al., 2008). When comparing site-specific genotype data, we discovered that L444P was more common in the cohort collected in Umeå in northern Sweden where 4.11% of the PD patients were heterozygous for L444P as compared with 0.79% of the patients in Stockholm, 2.47% in Gothenburg, and 1.72% in Lund. In total, 26 of the identified L444P carriers (68%) were from the northernmost Swedish provinces (Norrbotten and Västerbotten). Considering the skewed geographical distribution of L444P carriers in favor of Norrbotten and Västerbotten, and the elevated incidence of GD type III in the north provinces, we decided to analyze genotype data from southern Sweden (Gothenburg, Linköping, Stockholm, and Lund) and northern Sweden (Umeå) separately (Dahl et al., 1990). In performing a separate analysis for these 2 groups, we further avoid any bias that might be introduced in our analysis by the genetic population stratification that occurs in the Swedish population between northern and southern counties (Humphreys et al., 2011). The E326K variation was in HWE in controls from southern Sweden but not in controls from northern Sweden. Because of the lack of homozygous mutation carriers, we could not perform HWE analysis for N370S and L444P.

Statistical analysis showed that L444P was significantly associated with PD in southern Sweden (Table 2). L444P had an OR of

Table 2
Genotype frequencies and test statistics of E326K, N370S, and L444P

SNP	Genotype	Southern Sweden					Northern Sweden				
		CTRL, *n* (%)	PD, *n* (%)	χ^2 (df)	OR (CI)	p_c-value	CTRL, *n* (%)	PD, *n* (%)	χ^2 (df)	OR (CI)	p_c-value
E326K	CC	1731 (96.65)	898 (94.83)	4.93 (1)	1.57 (1.07–2.32)	0.081	141 (95.92)	552 (92.93)	NA	1.79 (0.75–4.29)	0.78
	CT/TT	59 (3.35)	48 (5.17)				6 (4.08)	42 (7.07)			
N370S	TT	1768 (99.89)	971 (99.28)	5.29 (1)	6.73 (1.32–30.75)	0.065	152 (100)	595 (99.50)	NA	1.79 (0.09–34.92)	1
	TC/CC	2 (0.11)	7 (0.72)				0	3 (0.50)			
L444P	AA	1812 (99.89)	971 (98.98)	10.27 (1)	9.33 (2.04–42.69)	0.0039	151 (99.34)	583 (95.89)	NA	6.49 (0.87–48.28)	0.13
	AG/GG	2 (0.11)	10 (1.02)				1 (0.66)	25 (4.11)			

Southern Sweden comprises the geographic areas of Stockholm, Gothenburg, Linköping, and Lund; Northern Sweden comprises samples collected at Umeå University Hospital.
Key: CI, 95% confidence interval; CTRL, control individuals; df, degrees of freedom; NA, not applicable; OR, odds ratio; p_c-value, *p*-value after Bonferroni correction for multiple testing; PD, Parkinson's disease; SNP, single nucleotide polymorphism.

Table 3
Meta-analysis of the southern and northern Swedish populations

SNP	p_c-value	Pooled OR	95% CI	Q	I^2
E326K	0.026	1.60	1.16–2.22	0.99	0.00
N370S	0.068	5.04	1.10–23.04	0.41	0.00
L444P	0.0020	8.17	2.51–26.63	0.77	0.00

Key: 95% CI, 95% confidence interval; I^2, I^2 heterogeneity index; OR, random-effects meta-analysis odds ratio; p_c-value, random-effects meta-analysis *p*-value with Bonferroni correction for multiple testing; Q, *p*-value for Cochran's Q statistic; SNP, single nucleotide polymorphism.

9.33, with a 95% confidence interval (95% CI) of 2.04–42.69, and a *p*-value after Bonferroni correction for multiple testing (p_c) of 0.0039. Genotype analysis of N370S and E326K showed a trend for association with PD in southern Sweden (Table 2). N370S had an OR of 6.73; 95% CI, 1.32–30.75, and a crude *p*-value of 0.022, but the association did not remain significant after correction for multiple testing, $p_c = 0.065$. The corresponding values for E326K were OR= 1.57, 95% CI: 1.07–2.32, *p*-value= 0.027, which also lost significance after Bonferroni correction. Allele analysis did not add substantial information (data not shown) with the exception of confirming the association between the minor allele (C) of N370S and an increased risk of developing PD (OR =7.27; 95% CI, 1.54–34.26; $p_c = 0.028$).

In the northern Swedish case-control material, all 3 *GBA* variants were overrepresented, and in particular L444P, which was present in 4.11% of the PD patients, as compared with 1.02% of the PD patients in southern Sweden. Statistical testing in this patient group was inconclusive, probably because of the low number of controls (Table 2). However, L444P showed a trend for association with an OR of 6.49 and a *p*-value of 0.042, but the CI overlaps 0 (95% CI, 0.87–48.28), and the *p*-value did not hold for Bonferroni correction ($p_c = 0.13$).

The distribution of mutation carriers was equal between sexes; 24 female carriers and 26 male carriers. Five healthy individuals were discovered to carry N370S or L444P mutations. Three of these individuals (2 heterozygous for N370S and 1 for L444P) were anonymous blood donors, for whom future development of PD cannot be excluded. The remaining 2 were healthy individuals heterozygous for L444P, with no signs of PD when followed up to ages 66 and 75 years.

The importance of these 3 variants in PD etiology in the overall Swedish population was evaluated using a meta-analysis. Cochran's Q statistics were nonsignificant and heterogeneity was estimated nonexistent, indicating that the results from these 2 studies are consistent and can be compared (Table 3). Data were analyzed under a random-effects model because we expected the effect of these mutations to vary between southern and northern Sweden. Pooled estimates confirmed the association between L444P and E326K and increased risk of PD, with a small effect size for E326K (OR 1.60; 95% CI, 1.16–2.22, $p_c = 0.026$) and a large effect size for L444P (OR 8.17; 95% CI, 2.51–26.63, $p_c = 0.0020$; Table 3). We also found a trend for association between N370S and PD with a *p*-value of 0.023, which did not remain significant after correction for multiple testing (OR 5.04; 95% CI, 1.10–23.04; $p_c = 0.068$).

3.2. Clinical characteristics of mutation carriers

We furthermore investigated the phenotype of patients carrying L444P and N370S mutations (Table 4). In concert with previous reports on PD patients with *GBA* mutations, we observed that many patients had a relatively early onset (50% were aged ≤55 years), in particular individuals carrying L444P mutations, who had an average age of onset of 55.7 ± 10.9 years, $p = 0.0052$ as compared with 709 of the noncarrier patients (for whom information on age of onset was available). N370S patients had an average age of onset of 63.6 ± 12.2 years, which is not lower than that of the noncarrier group ($p = 0.36$). Apart from the age of onset, mutation carriers did not differ significantly from other PD patients, 24.4% had a known familial history of PD and there were no other features indicating a more severe or a more rapidly progressing phenotype based on the clinical information available. Information on cognitive decline was available for a subset of affected carriers ($n = 34$) which allowed us to estimate that around 20% of the mutation carriers suffer from cognitive decline.

4. Discussion

Here, we describe a genetic study on 2 rare *GBA* mutations and 1 common *GBA* SNP in the largest Swedish PD case-control study reported to date. We found known pathogenic *GBA* mutations predominantly in PD patients. In a meta-analysis, comprising patients from the entire country, L444P strongly associated with PD, whereas N370S showed a trend for association. The allele association found between the minor allele of N370S and increased risk for PD in southern Sweden further supports the importance of N370S also in our study population. Both mutations were associated with high ORs, and somewhat broad 95% CIs clearly separated from 0. We also found an association between E326K and PD, in agreement with previous observations. The allele frequency for E326K in PD described by others corresponds to our observation in the Swedish population (Lesage et al., 2011). Because of the suggested role of E326K as a modifier (Chabas et al., 2005; Montfort et al., 2004), it would be interesting to investigate the possible combined effect of carrying this SNP plus one of L444P or N370S, but allele frequencies of these 3 mutations were too low to allow for haplotype analysis. Only 1 of the subjects in this study carried more than 1 mutated *GBA* allele. This individual was heterozygous for E326K and L444P and, with an age of onset 55 years, is not exceptional, considering *GBA* mutation carriers overall have relatively early onset of symptoms (Gan-Or et al., 2008; Nichols et al., 2009).

The common variant E326K presented a lower OR ($OR_{E326K} = 1.60$) than the rare mutations ($OR_{N370S} = 5.04$ and $OR_{L444P} = 8.17$), which was also expected considering the detrimental effect of N370S and L444P on enzyme activity. The effect of L444P was stronger than that of N370S, which is in agreement with the classification of the L444P mutations as severe, whereas N370S is classified as mild (Gan-Or et al., 2015; Mao et al., 2013).

The 2 mutations analyzed, L444P and N370S, were observed more often in PD patients than in control subjects. N370S was found in 10 patients (0.63%), 1 of which was homozygous, and 2 were controls (0.10%), whereas L444P was found in 35 patients (2.20%) and 3 control individuals (0.15%). Although individuals carrying homozygous *GBA* mutations usually suffer from GD, the homozygous N370S mutation carrier had a clear idiopathic PD diagnosis with no signs of dementia, when followed up at the age of 75. Homozygous *GBA* mutation carriers with typical PD phenotypes have also been reported by others (Lesage et al., 2011). The overall occurrence of these 2 *GBA* variations was 2.77% in the Swedish PD population, which is comparable with what has been reported globally (around 3% in PD cohorts without Ashkenazi Jewish ancestry), although this number is very different depending on ethnicity (Sidransky et al., 2009). In Norway, the frequencies of L444P and N370S are also somewhat lower than 3%, indicating that these variations are not very frequent in the Scandinavian populations (Sidransky et al., 2009; Toft et al., 2006). We observed a clear stratification in the Swedish population, with a higher mutation load in the northern parts of the country. In total, 29 of the 50 identified *GBA* mutation carriers (controls and patients) were from northern Sweden. Of these, 26 individuals were heterozygous for

Table 4
Characteristics of PD patients with *GBA* mutations

Genotype	Origin	Gender	Heredity	Age, y	Age at onset, y	Disease duration, y	Hoehn and Yahr	Cognitive decline
N370S +/+	Gothenburg	F	N	69	66	3	NA	N
N370S +/−	Gothenburg	F	N	66	54	12	NA	NA
N370S +/−	Linköping	M	Y	74	68	6	NA	NA
N370S +/−	Lund	F	N	79	70	9	5	Y
N370S +/−	Stockholm	F	N	56	45	11	3	NA
N370S +/−	Stockholm	M	N	80	78	2	4	NA
N370S +/−	Stockholm	F	N	86	85	1	3	NA
N370S +/−	Umeå	M	N	47	43	4	2	N
N370S +/−	Umeå	M	N	71	61	9	2	N
N370S +/−	Umeå	M	N	71	66	4	3	Y
L444P +/−, E326K +/−	Gothenburg	M	N	66	55	11	NA	NA
L444P +/−	Gothenburg	M	N	61	61	1	NA	NA
L444P +/−	Gothenburg	M	Y	76	67	9	NA	NA
L444P +/−	Gothenburg	F	Y	60	52	8	NA	NA
L444P +/−	Lund	F	N	64	57	7	3	Y
L444P +/−	Lund	M	Y	63	68	5	3	Y
L444P +/−	Stockholm	F	N	64	50	14	2	NA
L444P +/−	Stockholm	M	N	54	50	4	2	NA
L444P +/−	Stockholm	F	Y	76	75	1	NA	N
L444P +/−	Stockholm	M	N	83	73	10	1	N
L444P +/−	Umeå	M	N	59	43	16	4	Y
L444P +/−	Umeå	M	Y	67	65	2	2	N
L444P +/−	Umeå	M	N	44	40	4	2	N
L444P +/−	Umeå	F	N	72	65	7	3	Y
L444P +/−	Umeå	F	N	66	63	3	3	N
L444P +/−	Umeå	F	N	59	48	10	3	N
L444P +/−	Umeå	M	Y	55	41	13	4	N
L444P +/−	Umeå	M	N	55	42	13	2	N
L444P +/−	Umeå	M	Y	48	46	2	2	N
L444P +/−	Umeå	F	N	52	41	10	2	N
L444P +/−	Umeå	F	N	58	52	5	2	N
L444P +/−	Umeå	M	N	62	61	1	2	N
L444P +/−	Umeå	M	Y	47	41	6	2	N
L444P +/−	Umeå	F	N	54	53	2	1.5	N
L444P +/−	Umeå	F	Y	75	72	3	2	N
L444P +/−	Umeå	F	N	58	49	8	2.5	N
L444P +/−	Umeå	F	N	67	57	9	2.5	N
L444P +/−	Umeå	F	Y	62	50	11	3	Y
L444P +/−	Umeå	M	N	59	61	2	2	N
L444P +/−	Umeå	F	N	61	62	1	2	N
L444P +/−	Umeå	F	N	47	43	3	2	N
L444P +/−	Umeå	F	Y	62	45	16	3	N
L444P +/−	Umeå	M	Y	65	55	10	2	N
L444P +/−	Umeå	M	N	83	80	2	2	N
L444P +/−	Umeå	M	N	71	68	2	2	N

Key: F, female; GBA, glucocerebrosidase; M, male; N, no; NA, data not available; PD, Parkinson's disease; Y, yes.

L444P, which corresponds to 4.11% of the PD patients in northern Sweden, as compared with 1.02% in southern Sweden. Only 3 individuals from northern Sweden were heterozygous for N370S, which was comparable with the percentage of heterozygous individuals in the rest of the population. These data are consistent with the increased prevalence of Norrbottnian type GD in northern Sweden (Norrbotten and Västerbotten), which has been linked to homozygosity of the L444P mutation (Dahl et al., 1990).

Because our study did not include all pathogenic *GBA* variations discovered, we might be overlooking important components of the *GBA* contribution to PD pathology in Sweden. It is possible that other *GBA* mutations, known or yet to be discovered, are more predominant in the Swedish PD population. On the other hand, our results suggest that *GBA* mutations contribute to the high incidence of PD reported from northern Sweden, but only constitute a rare risk factor for PD elsewhere in Sweden (Linder et al., 2010). Our study is further limited by the uneven distribution of controls between southern and northern Sweden and by the unknown age and specific characteristics of a large number of control subjects (n = 855) who were blood donors and can, therefore, be expected to have a lower mean age at enrollment than the PD patients in this study.

In agreement with previous studies, we found that L444P mutation carriers have lower age of onset of PD symptoms than noncarriers (p = 0.0052). A few controls were found to carry *GBA* mutations (0.25%), 3 were anonymous blood donors and they might all be at risk for developing PD in the future. Finding carriers of pathogenic mutations among controls is not unexpected, and healthy control subjects with a copy of the N370S or L444P allele have been reported in other populations as well (Sidransky et al., 2009; Toft et al., 2006). Moreover, the penetrance of the L444P mutation has been estimated to be 30% at the age of 70 (Anheim et al., 2012). It is possible that the L444P carriers in our study (aged 66 and 75) will develop PD with increasing age, but because one of the hallmarks of *GBA* mutations is early disease onset, we expect these individuals to remain healthy. Healthy mutation carriers are interesting subjects for further genetic studies, as they might carry protective genetic variants, either generally neuroprotective or counteracting the effect of lower GCase activity.

It is not clear how mutations in the *GBA* gene affect PD pathogenesis, but there is an interesting connection to another PD candidate gene, *SNCA*, which strengthens the hypothesis of involvement of the endosomal and/or lysosome-autophagy pathways in PD. For example, the membrane-bound forms of

α-synuclein and GCase have been demonstrated to interact under lysosomal conditions, leading to inhibition of GCase (Yap et al., 2011, 2013). The levels of α-synuclein protein are affected by mutations in *GBA* both in cell culture and in vivo in *GBA* transgenic mice (Cullen et al., 2011; Mazzulli et al., 2011). Similarly, knockdown of *GBA* results in increased α-synuclein levels (Mazzulli et al., 2011). It should be noted that there are also data reporting no effect on α-synuclein or lysosomal function of GCase inhibition (Dermentzaki et al., 2013). However, untreated GD patients have increased plasma levels of oligomeric α-synuclein, whereas such an increase was not observed in GD patients having received GCase replacement therapy (Pchelina et al., 2014). Inaccurate GCase handling is hypothesized to result in accumulation of α-synuclein (Mazzulli et al., 2011). The elevated α-synuclein levels observed as a result of low GCase activity would then lead to further reduction in GCase activity and impairment of the intracellular trafficking of GCase, which becomes retained in the endoplasmic reticulum (ER; Mazzulli et al., 2011). Interestingly, α-synuclein accumulation can interfere with vesicle transport between the ER and the Golgi apparatus (Cooper et al., 2006). Furthermore, *GBA* mutations might impair the unfolded protein response of the ER (Kurzawa-Akanbi et al., 2012). An alternative explanation suggests that *GBA* deficiency impairs autophagy and, thereby, results in α-synuclein accumulation (Du et al., 2015). Altered expression of proteins involved in autophagy has been observed as a result of impaired GCase activity (Du et al., 2015; Rocha et al., 2015). Moreover, α-synuclein accumulation can be reversed by the use of drugs inducing autophagy in cell culture (Cullen et al., 2011; Du et al., 2015).

5. Conclusions

This genetic study has been conducted on the largest PD case-control material yet reported from Sweden. We found N370S and L444P predominantly in PD patients and they were significantly associated with PD in Sweden. Both mutations are expected to contribute to PD pathology. How heterozygous *GBA* mutations affect cellular mechanisms and contribute to PD pathogenesis is unknown, but accumulating evidence suggest the mechanism involves α-synuclein accumulation and impairment of the lysosome and autophagy pathways. L444P mutations were more common in northern Sweden, which is consistent with the higher incidence of GD type III in this part of Sweden. The common variant E326K was also found to associate with increased risk for PD in Sweden, supporting a role for common genetic variations in PD etiology. Further genetic screening and studies of *GBA* should bring new insights into the pathophysiology of PD, which in turn may result in earlier diagnosis and design of relevant therapeutic strategies.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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